

IN THE CLAIMS:

1. (Currently Amended) An *in situ* fluorescence method for external on-line monitoring of the physiological state of a sulfur-deprived algal culture inside a closed photobioreactor system to ascertain ~~its~~ the culture ability to produce H₂ under sulfur depletion, comprising:

- a) providing a sample of sulfur-deprived algal culture containing photosynthetic components;
- b) illuminating said sample with artificial or natural illumination;
- c) determining the onset of H₂ photoproduction by measuring the percentage of H₂ in a produced gas phase at multiple times to ascertain the point immediately after the anerbiosis subsequent to the physiological phases of O₂ production and O₂ consumption sequence to obtain data of regarding H₂ production as a function of time; and
- d) determining any abrupt change in the following three *in situ* fluorescence parameters:

- i) an abrupt increase in F_i (the steady-state level of chlorophyll fluorescence in light adapted cells);

- ii) an abrupt decrease in F_m' (the maximal saturating light induced fluorescence level in light adapted cells); and'

- iii) a precipitous and abrupt decrease in $\Delta F/F_m' = (F_m' - F_i)/F_m'$ [where $\Delta F = F_m' - F_i$] (the calculated photochemical activity of photosystem II (PSII)) that ~~signal~~ signals the full reduction of the plastoquinone pool between PSII and PSI, which indicates the start of anaerobic conditions that in turn induces the synthesis of the hydrogenase enzyme required for subsequent H₂ production, and thereafter slowing down of the abrupt decrease and partial recovery of ~~$\Delta F/F_m'$ signal~~ $\Delta F/F_m'$ signals at least partial oxidation of the plastoquinone pool as the main factor to regulate H₂ production under sulfur depletion.

- 2. (Original) The method of claim 1 wherein said algal culture is any oxygenic photosynthetic microorganism that has a hydrogenase.

3. (Original) The method of claim 2 wherein said oxygenic photosynthetic microorganism that has a hydrogenase is green algae.
4. (Currently Amended) The method of claim 3 wherein said green algae is selected from the group consisting of *Chlamydomonas reinhardtii*, *reinhardtii*, *Scenedesmus obliquus* and *Chlorella vulgaris*.
5. (Original) The method of claim 4 wherein said green algae is *Chlamydomonas reinhardtii*.
6. (Original) The method of claim 5 wherein said abrupt increase in F_t is determined using a fluorometer employing a weak modulated pulse-probe fluorescence method.
7. (Original) The method of claim 5 wherein said *in situ* measurement of fluorescence is at or about $\lambda > 710$ nm .
8. (Original) The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with an optical fiber probe affixed onto a surface of an illuminated glass containing fluorescence excited sample or alternatively with a lens system.
9. (Currently Amended) The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with a fluorometer set close to the edge of the a bioreactor.
10. (Original) The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with a lens set close to the edge of the bioreactor.
11. (Currently Amended) The method of claim 8 wherein a saturated actinic excitation pulse is applied on top of said a weak modulated probe pulse.
12. (Currently Amended) The method of claim 11 wherein said saturated actinic excitation pulse is a 0.8 s pulse (about $\lambda < 710$ nm, $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-2}$ PAR) from an 8 V/20W halogen lamp.
13. (Original) The method of claim 11 wherein actinic light is about 655nm, $250 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR from a LED array for about 2 s for fluorescence induction.
14. (Original) The method of claim 12 wherein said saturating actinic excitation pulse is applied on top of a weak modulated probe that flashes at about 3 μs pulses from a 655 nm light-emitting diode at frequencies of from about 600 Hz or 20 kHz.

15. (Original) The method of claim 14 wherein efficiency of photochemical conversion of absorbed light energy in PSII is calculated after dark adaptation, where $F_v/F_m = (F_m - F_0)/F_m$.
16. (Original) The method of claim 14 wherein efficiency of photochemical conversion of absorbed light energy in PSII is calculated under steady-state actinic light illumination, where $\Delta F/F_m' = (F_m' - F_0)/F_m'$.